NO (Nitric Oxide) Plus Detection Kit

RUO *Research Use Only* REF *21023* Σ *≤ 1000 assay* 2 ℃ 8 ℃

PRODUCT FEATURES

- **NO (Nitric Oxide) Plus Detection Kit** is designed for the effective measurement of Nitric Oxide from various samples.
- Effective nitric oxide measurement purely ensures accuracy and repeatability from indirectly determining of NO^-.
- The fast, simple procedure for measurement suitable Nitric Oxide assay.

INTRODUCTION

Nitric oxide (NO^-) is radical compound produced during the transformation of L-arginine to citrulline by nitric oxide synthase (NOS). Nitric oxide functions as the effecter molecule for macrophage-mediated cytotoxicity and has also been recognized to inhibit mitochondrial respiration and DNA synthesis. Nitric oxide recently raised a lot of interests as a suppressive factor that inhibits anti-tumor immune response.

The **NO (Nitric Oxide) Plus Detection Kit** based on diazotization (Griess method) assay can detect in vitro NO^- concentration. The kit enables researchers to overcome difficulties in detecting NO^- concentration due to the short half life (about 5 seconds) of gaseous nitric oxide. The kit will accurately detect the concentration of NO^- by indirectly measuring nitrite (NO_2^-), which is by-product of nitric oxide transformation in living tissue. The kit is based on the colorimetric change, which occurs when naphthylethylenediamine is added to the by-product of reaction between sulfanilamide and nitrite. The Kit is suitable for quantitative measurement of total NO production easily by using two solution.

APPLICATION

NO assay using the NO (Nitric Oxide) Plus Detection Kit is suitable for use in a indirectly determining of NO_2^- :

- ✓ Quantitative analysis of total nitric oxide
- ✓ Application to culture supernatant, Plasma, Serum, Saliva, Urine, etc.
- ✓ Research for cell cytotoxicity and immune response

KIT CONTENTS

Components	≤ 1000 assay
N1 buffer (Sulfanilamide in the reaction buffer)	50 ml
N2 buffer (Naphthylethylenediamine in the stabilizer buffer)	50 ml
Nitrite (FW: 69.0) standard, 2 mM	1 ml x 2 vial

STORAGE CONDITION

The NO (Nitric Oxide) Plus Detection Kit can be stored at 4 ℃. Under these conditions, the kit can be stored for up to 6 months without any reduction in performance and quality.
If the product is unopened, it can be stored at 4 ℃ for up to 1 year.

IMPORTANT NOTES

1. When N1 buffer stores in a refrigerator, there could be little amount of sediments. In the presence of sediments, please dissolve the sediments with vortexing at RT.
2. When collecting supernatant from the cultured cells, be careful not to include cell debris. The following steps are recommended to eliminate deviation. First, harvest cells by centrifuging. Second, perform a filtration using 0.22 μm filter.
3. You may have to prepare a reference curve with the Nitrite standard solution to ensure accurate NO_2^- amount, using the same buffers or media used for experimental samples. The different levels of sensitivity may be achieved in different buffers or media.
4. If the samples contain over 1mM of nitrite, the reaction color may initially become dark pink. In this case, you must dilute the samples with the same buffers or media.
5. In measurement of absorbance, you can use a plate reader with a filter between 520-560 nm within 20-30 min.

ADDITIONAL REQUIREMENTS

1. UV-Spectrophotometer
2. 96 well Plate

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

At iNtRON we pride ourselves on the quality and availability of our technical support. Our CRT center is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology in the use of iNtRON products. If a iNtRON product does not meet your expectations, simply call your local distributor. If you have questions about product specifications or performance, please call iNtRON Technical Services or your local distributor.

NOTICE BEFORE USE

NO (Nitric Oxide) Plus Detection Kit is intended for Research Use Only. This product is not intended for the diagnosis, prevention, or treatment of disease. All care and attention should be exercised in the handling of the products. Do not use internally or externally in humans or animals. Please observe general laboratory precaution and utilize safety while using this kit.

PROTOCOL I – Nitrite standard curve

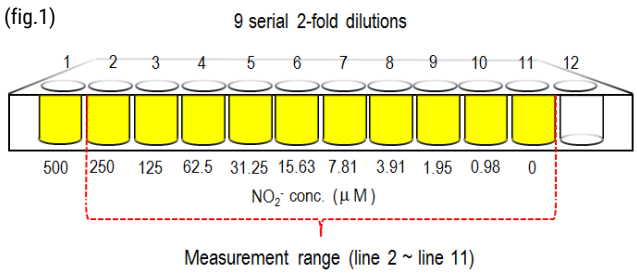
❖ Designate triplicates in the 96 well plate for the nitrite standard curve.

1. Dispense 100 μl of ultra pure water into wells in line 2-11 (or rows B-H).

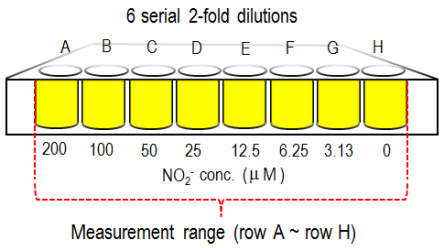
2. Prepare nitrite standard solution for line 1[A] (or row A)[B].
   A) line 1 - Add D.W 150 μl + nitrite standard (2 mM) 50 μl. (Top conc. 500 μM)
   B) row A - Add D.W 180 μl + nitrite standard (2 mM) 20 μl. (Top conc. 200 μM)

3. Perform 9 serial 2-fold dilutions (fig.1) or 6 serial 2-fold dilutions (fig.2) in triplicate to generate the Nitrite Standard reference curve, discarding 100 μl from the 0.98 μM or 3.13 μM set of wells, respectively.
   And, for accurate measurement of standard curve, do not add any nitrite solution to the last set of line 11 and row H wells. The final volume in each well is 100μl.

(fig.1)



(fig.2)



4. Induce pre-reaction by adding 50 µl of N1 buffer to the samples. And incubate for 5-10 min at room temperature.

5. Carry out final reaction by adding 50 µl of N2 buffer.

6. Leave the solution for 10 minutes in room temperature, and measure the absorbance value between 520-560 nm using a plate reader.
Caution : For determining accurate values, measure from line 2 to 11 for 9 serial dilution methods (fig.1) and from row A to H for 6 serial dilution methods (fig.2).

7. According to above absorbance, you may generate a Nitrite Standard reference curve. Plot the average absorbance value of each concentration of the Nitrite Standard as a function of "Y" with nitrite concentration as a function of "X".

PROTOCOL II– Sample measurement

1. Collect about 100 µl of samples, and add the samples to wells in duplicate or triplicate.
Note : When collecting supernatant from the cultured cells, be careful not to include cell debris. The following steps are recommended to eliminate deviation. First, harvest cells by centrifuging. Second, perform a filtration using 0.22 µm filter.

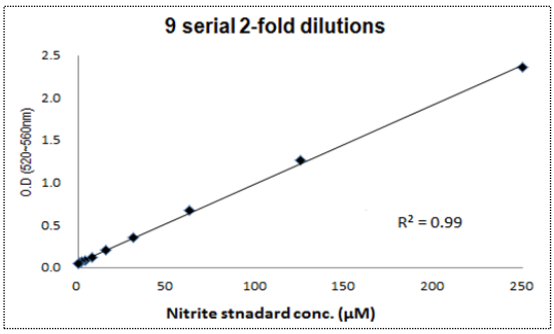
2. Induce pre-reaction by adding 50 µl of N1 buffer to the samples. And incubate for 5-10 min at room temperature.

3. Carry out final reaction by adding 50 µl of N2 buffer.

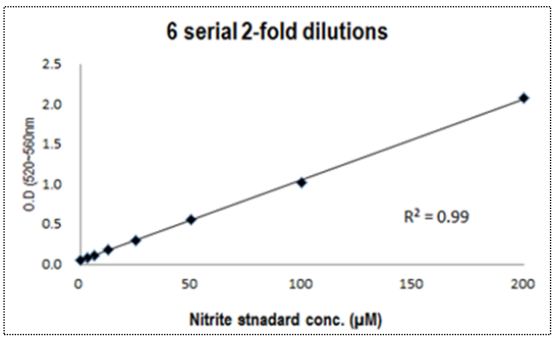
4. Leave the solution for 10 minutes in room temperature, and measure the absorbance value between 520-560 nm using a plate reader. You may now calculate the nitrite concentration based on standard curve formula.

TECHNICAL INFORMATION

❖ The 9 serial 2-fold dilutions nitrite standard curve.



❖ The 6 serial 2-fold dilutions nitrite standard curve.



TROUBLE SHOOTING GUIDE

Problem	Possible Cause	Recommendation
Reaction wells become dark pink.	The measurement range is exceeded.	• Samples contain over 1 mM of nitrite. • In this case, you must dilute the samples with the same buffers or media.
	Nitrite standard curve slope value error.	• Dilute the nitrite standard solution to the protocol and retest. • The closer the slope value (R^2) is to$\leq$ 1, the more accurate than the measured value.
No color change in Nitrite standard test.	Reaction buffer not added.	• Refer to the above protocol and add the buffer according to each step, and proceed the experiment.

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